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Changes in Post-anthesis Assimilates in Stem and Spike Components of Italian Ryegrass (*Lolium multiflorum* Lam.). I. Water Soluble Carbohydrates*

STEPHEN M. GRIFFITH

USDA ARS, National Forage Seed Production Research Center, Oregon State University, Corvallis, Oregon 97331, USA

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Stem carbohydrate reserves, in ryegrass grown for seed, may play a vital role in maintaining seed growth, especially under conditions of limited photosynthesis. Little is known concerning the processes controlling stem carbohydrate utilization and partitioning in ryegrass with respect to seed growth. The objective of this investigation was to determine detailed post-anthesis changes in stem and spikelet carbohydrates as affected by modification of source and sink strength. Source–sink relations were altered by imposing detillering or detillering-defoliation treatments at anthesis. Patterns of carbohydrate distribution of the ryegrass stem were different, both among positions within the stem and with age. Stem carbohydrates accumulated during early stages of seed growth and then declined as seeds matured. Reducing sugars comprised only a small fraction of the stem's total water soluble carbohydrates. Detillering induced the formation of new tiller sinks, thus increasing sink strength and reversing the carbohydrate gradient from spikelet (seed) sinks to new tiller sinks. Defoliation, combined with detillering, decreased source strength by reducing total stem carbohydrate. In control plants, carbohydrate levels appeared adequate to support maximum seed set, whereas conditions for reduced carbohydrate levels, resulting from detillering or detillering plus defoliation, lowered seed set. Results suggest that under conditions of limited source strength (e.g. reduced photosynthetic capacity), the stem plays a major role in partitioning assimilates to compensate for sink demand. New tiller growth during the period of seed development may out-compete seeds for available carbohydrate and thus reduce seed set.

Key words: Assimilate partitioning, storage, remobilization, stem, spike, seed.

INTRODUCTION

It is well established that grass stems function in accumulating and remobilizing reserve assimilates. The major carbohydrate reserve in stems of temperate grasses is fructan (Pollock and Chatterton, 1988). Starch levels are low or absent in temperate grass stems (Hendrix, 1985). Following anthesis, contribution of stem C-assimilates to final seed yield has been reported to range from 10 to 25% in wheat and to 80% for barley (Borrell *et al.*, 1989). Mobilization of wheat stem reserves may permit the grain to attain higher growth rates, particularly when photosynthesis (source strength) becomes limiting (Fisher, 1983).

More information is needed to better assess the stem's role in providing assimilate for seed growth in ryegrass under conditions which may limit assimilate supply. Few detailed reports exist in the literature which characterize and quantify stem carbohydrate assimilates in temperate herbage grass stems from anthesis to seed maturity. Numerous reports have studied patterns of carbohydrate partitioning in grasses grown for herbage but not for seed. Patterns of ^{14}C partitioning in reproductive swards of ryegrass managed for a seed crop have been described (Ong, Colvill and Marshall, 1978; Clemence and Hebblethwaite, 1984; Colvill and Marshall, 1984), but their findings only describe changes in tissue ^{14}C radioactivity and do not quantify changes in ^{14}C-labelled or unlabelled assimilate pools. This information is vital to further assess source–sink strength and the dynamics of assimilate partitioning to seeds.

The objective of this investigation was to determine post-anthesis changes in the lower, middle and upper stem, and spikelet carbohydrates as affected by modified source and sink strengths. Effects on seed yield components were also determined.

MATERIALS AND METHODS

Plant growth

Single plants of Italian ryegrass (*Lolium multiflorum* Lam. cv. Marshall) were grown in black plastic cones, containing 450 cm^3 washed sand, in a glasshouse. Each cone received 200 ml of 25 ppm N (Peters 20:20:20) on alternate days. Supplemented light using high pressure sodium vapour lamps, with an average quantum flux density of 500 $\mu E\ m^{-2}\ s^{-1}$, extended daylight to 16 h. Averaged daily maximum and minimum temperatures were 23 °C and 11 °C, respectively. Plant population density was 180 plants m^{-2} prior to anthesis, at which time uniform plants were selected for treatment and grown at 108 plants m^{-2}.

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Source–sink manipulation

Defoliation and defoliation-detillering treatments were imposed at anthesis (50% spikelets per spike at anthesis). Detillering was done by removing all tillers leaving only the main stem which had been identified and tagged at emergence from the boot. New tiller regrowth on detillered plants was allowed to proceed normally. Leaf defoliation was imposed by removing all blades and sheaths from the main stem.

Main stems were harvested at 0, 2, 4, 8, 16 and 32 d after anthesis (DAA) and subdivided into lower, middle and upper (peduncle and rachis) stem sections, and spikelets. All plant materials were dried at 70 °C for 48 h. Treatments were replicated four times.

Carbohydrate analysis

Dried stem and spikelet materials were ground using a Wiley Mill and passed through a 40 mesh (openings 1·57 mm^2) screen. Water soluble carbohydrates (WSC) were extracted from ground tissue in water at 75 °C for 2 h. Following extraction, the mixture was centrifuged at 14000 *g* for 20 min. The supernatant was either assayed directly for reducing sugars (RS) or hydrolysed in 1 N HCl, then assayed for RS to determine total WSC. Reducing sugars were quantified using the Nelson procedure (Hodge and Hofreiter, 1962). Sucrose plus fructan concentrations (WSC − RS), were calculated by subtracting RS from total WSC. The WSC − RS and RS were expressed on a d. wt and plant part basis.

RESULTS

Plant growth

During seed development (0–32 DAA), f. wt remained relatively constant in the lower, middle, and upper (peduncle and rachis) stem of greenhouse grown Italian ryegrass (*L. multiflorum* Lam.), although there appeared to be a slight decline in f. wt between 2 and 16 DAA (Fig. 1). The proportion of total f. wt among lower, middle and upper stem segments at 7 DAA was 54, 32 and 14%, respectively. No measurable change in spike f. wt was observed for the first eight DAA, but increased dramatically between 8 and 16 DAA. A reduction in spike f. wt between 16 and 32 DAA (seed maturity) followed. Final spike weight was 710 ± 30 mg.

Removing all tillers but the main stem at anthesis, reduced lower stem and spike f. wt by 16 DAA (Fig. 1). Detillering reduced final spike weight by 38% (440 ± 30 mg) compared to control. By 32 DAA, f. wt declined in the lower, middle, and upper stem by 61, 53 and 49%, respectively, compared to control. Combined defoliation and detillering lowered the f. wt of all stem segments and reduced final spike weight by 54% (340 ± 30 mg). Overall, the rate of spike weight gain declined as source limitation became greater.

Single-seed final d. wt did not significantly differ ($\alpha = 0{\cdot}05$ level) among control (3·46 ± 0·28 mg), detillered (3·40 ± 0·16 mg), and detillered-defoliated (3·21 ± 0·42 mg) treated plants, indicating that the reductions in final spike weight, resulting from detillering and detillering-defoliation treatment, reflected a decline in seed set. As a further note, detillering and detillering-defoliation treatments resulted in new tiller growth, in addition to the regrowth of decapitated tillers.

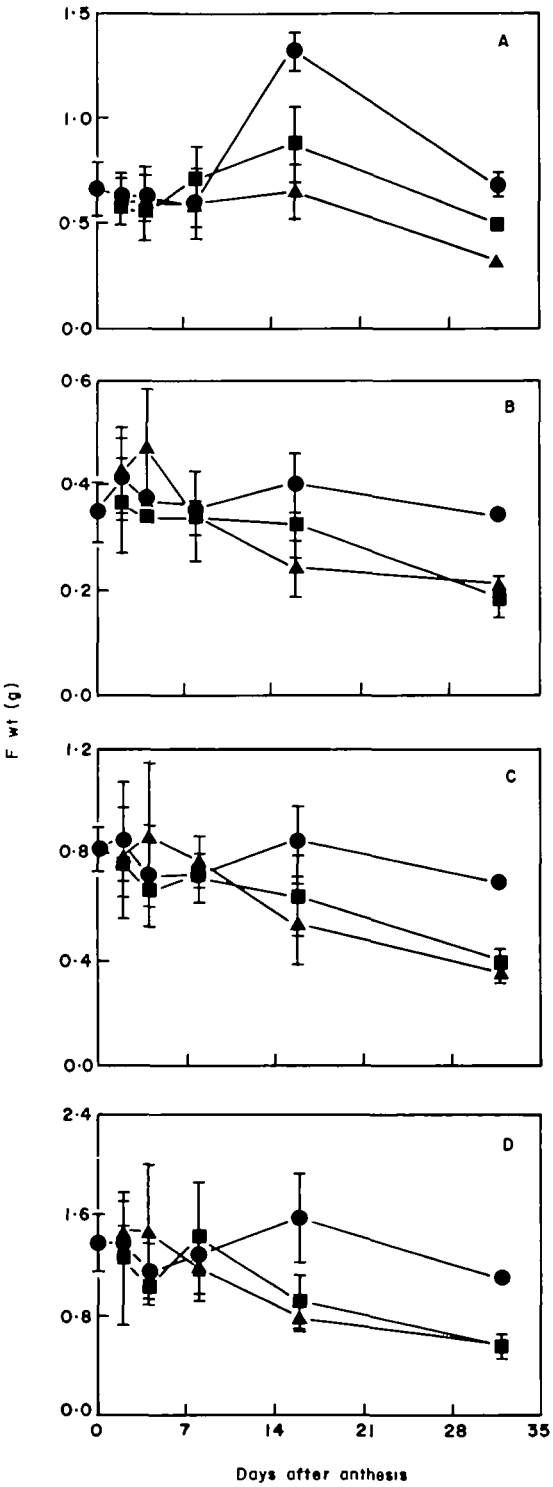

FIG. 1. Post-anthesis changes in f. wt in spike (A) and upper (B), middle (C), and lower (D) stem segments of control (●), detillered (■), and detillered-defoliated (▲) plants. Bars indicate ± s.e. mean.

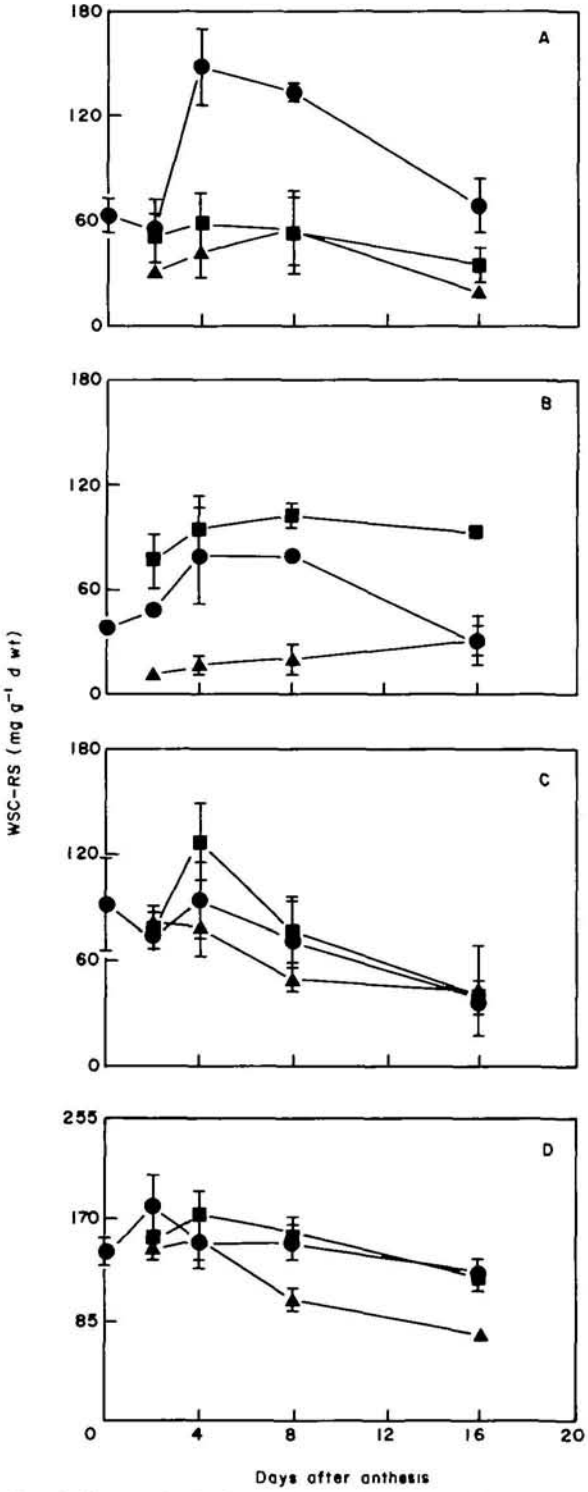

FIG. 2. Post-anthesis change in total water soluble carbohydrates (WSC) minus reducing sugars (RS), WSC – RS, in spike (A) and upper (B), middle (C), and lower (D) stem segments of control (●), detillered (■), and detillered-defoliated (▲) plants. Bars indicate ± s.e. mean.

Carbohydrates

In the control treatment, the WSC – RS fraction comprised about 77, 61 and 64% of the total WSC in the lower,

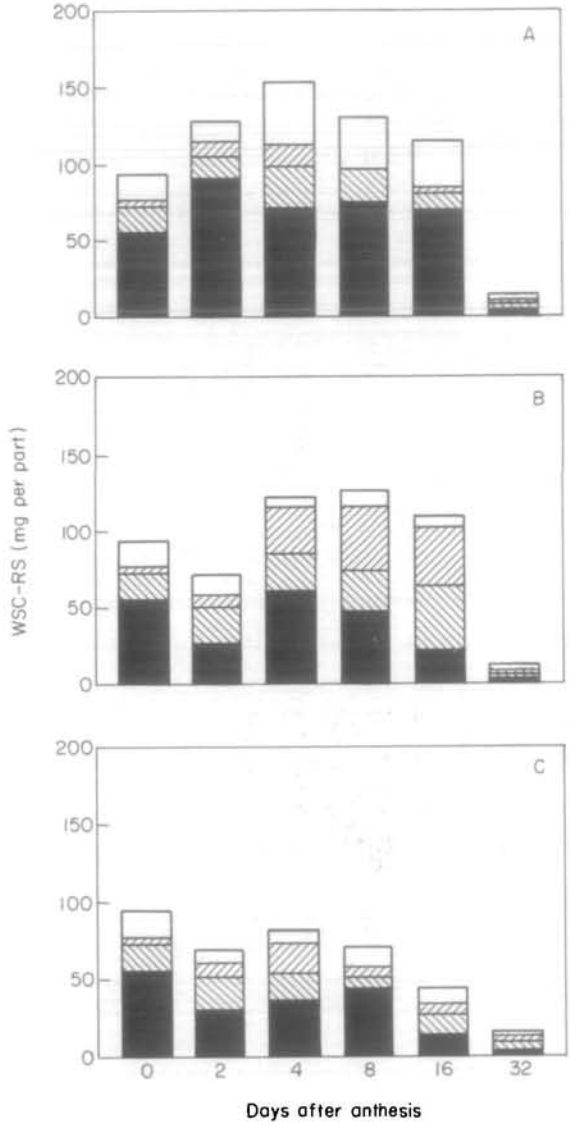

FIG. 3. Post-anthesis change in absolute water soluble carbohydrates (WSC) minus reducing sugars (RS), WSC – RS, among spike (▩) and upper (▨), middle (▧), and lower (■) stem segments of control (A), detillered (B), and detillered-defoliated (C) plants.

middle and upper stem, respectively. Thin-layer chromatographic analysis of unhydroylsed stem and spikelet total WSC, showed a complex of low- and high-molecular weight fructan polymers present, in addition to sucrose and reducing sugars (results in preparation). The WSC – RS was more concentrated in the lower stem and decreased acropetally (Fig. 2). The lower stem WSC – RS concentration remained nearly constant throughout the period of seed development. The middle stem WSC – RS concentration decreased between 8 and 32 DAA. The upper stem increased in WSC – RS concentration until 8 DAA and declined thereafter.

Concentrations of WSC – RS in the lower and middle stem were unaffected by detillering alone but the upper stem contained higher WSC – RS concentrations, which did not diminish with time (Fig. 2). Combined detillering-defoliation reduced WSC – RS concentrations in the lower and middle

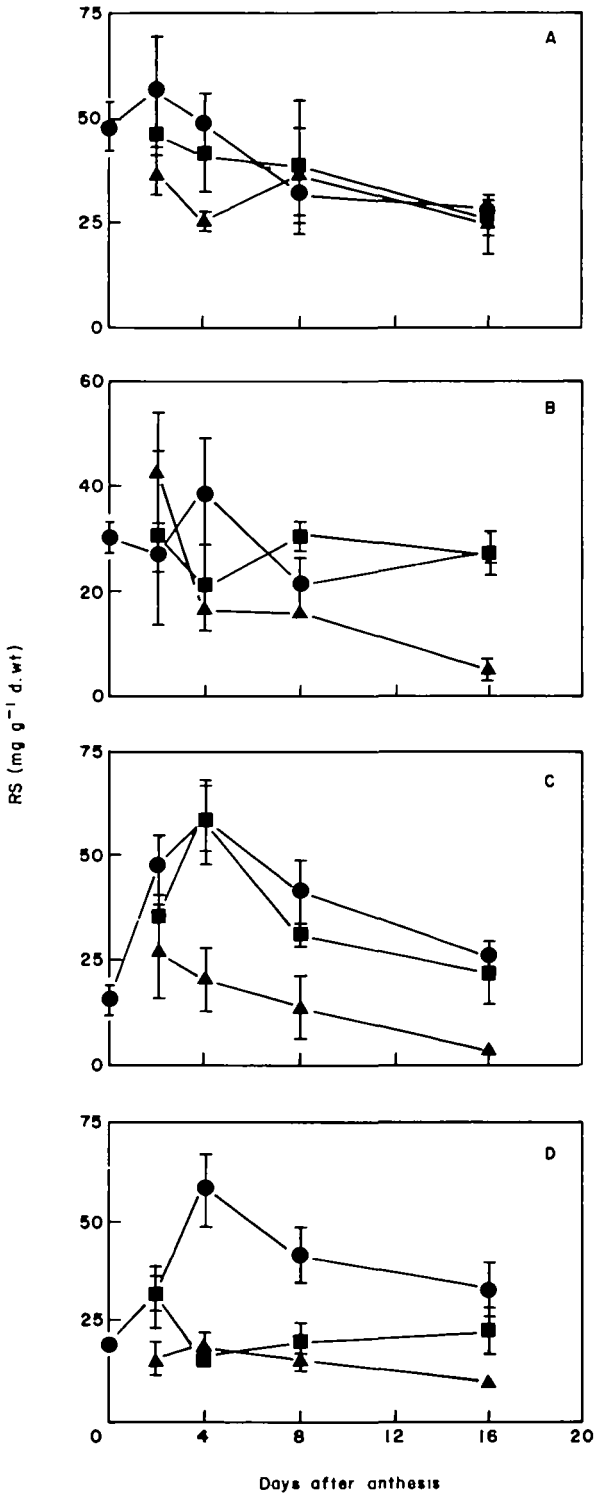

FIG. 4. Post-anthesis changes in reducing sugars in spike (A) and upper (B), middle (C), and lower (D) stem segments of control (●), detillered (■), and detillered-defoliated (▲) plants. Bars indicate ± s.e. mean.

stem segments; WSC – RS did not accumulate in the upper stem.

Control spike WSC – RS concentrations increased from 2 to 4 DAA (Fig. 2). At 4 DAA, WSC – RS comprised 75% of the total WSC, but declined thereafter. In both detillering and detillering-defoliation treatments, spike total WSC – RS concentration remained low and nearly unchanged compared to control.

Combined total stem and spike WSC – RS levels among control and detillered plants were nearly similar through the period of seed development (Fig. 3). Only the partitioning of WSC – RS among stem segments varied. Detillering resulted in greater carbohydrate partitioning from the lower stem to the upper stem, with total spike WSC – RS simultaneously declining. Detillering-defoliation treatment reduced WSC – RS levels in all stem segments and spike compared to control.

Reducing sugar in the lower, middle, and upper stem segments comprised only a small proportion of the total WSC. The pattern of RS accumulation was similar for all stem segments. Stem RS concentration reached a maximum by 4 DAA and then declined (Fig. 4). The RS concentration was greatest in the lower and middle stem regions, each with nearly equal concentrations of RS. The RS in the lower and upper stem did not increase in concentration as a result of detillering. All stem internodes of detillered-defoliated plants contained low RS concentrations compared to control.

Spike RS concentration was greatest during early seed development (Fig. 4), but declined to low values by 8 DAA. Both detillering and detillering-defoliation treatments lowered spikelet RS concentration during the first 4 DAA.

DISCUSSION

Results of this investigation give a detailed account of post-anthesis changes in carbohydrate among stem internodes and spikelets in Italian ryegrass (*L. multiflorum*) as affected by artificial source–sink manipulations. Findings of this study, with regard to stem carbohydrate accumulation, support previous published data for ryegrass (Pollock and Jones, 1979), and are similar to results reported for wheat (Borrell *et al.*, 1989). Further, this report expands the knowledge of the dynamics of stem WSC temporary storage and remobilization, compared to the few reports describing ^{14}C partitioning in reproductive swards of ryegrass grown for seed (Clemence and Hebblethwaite, 1984; Colvill and Marshall, 1984).

Patterns of stem WSC distribution in ryegrass changed both among positions within the stem and with continued seed growth. Soluble carbohydrates accumulated in stems during early stages of seed growth and then declined (Figs 2, 3 and 4). Reducing sugars comprised only a small fraction of the total stem WSC. At anthesis, the greatest concentration of reserve WSC was located in the lower two-thirds of the stem. Previous findings indicate that fructans probably comprise the major portion of this reserve (Pollock and Jones, 1979). Fructan polymers of varying sizes were present in stem and spikelet tissue.

Reproductive tillers of ryegrass are thought to be independent from adjacent tillers for carbohydrate supply, while young, less developed tillers are not (Marshall, 1985). Radiolabelled ^{14}C-pulse-chase studies have shown little export of ^{14}C occurring from the flowering main shoot of ryegrass to adjacent reproductive tillers (Colvill and

Marshall, 1981, 1984). Likewise, when conditions such as high moisture, light, or nitrogen fertilizer application, favour new tiller growth, reproductive tillers have been shown to remobilize basal stem carbohydrate reserves to new tiller sinks (Clemence and Hebblethwaite, 1984). Circumstantial evidence from this study supports these notions. In control plants, lower stem post-anthesis WSC remained nearly unchanged, but declined when tiller regrowth and new tiller initiation was stimulated by detillering. Furthermore, throughout seed filling control stems maintained a WSC − RS gradient in the direction of the spikelets; detillering reversed this gradient towards new tiller sinks. This may have caused the observed losses in seed set through assimilate deprivation to developing seeds. Low WSC − RS in the spikelet in detillered plants indicated that carbohydrate was redirected to the lower stem, rather than being used for seed growth. Collectively, these findings suggest that young tillers are strong sinks, capable of competing with developing seeds for available assimilates. In contrast, wheat with an annual growth habit which does not form new tillers as the result of detillering, increases grain and straw yield components upon detillering at anthesis (Mohamed and Marshall, 1979).

It appears that when factors favour new tiller growth, the tillers become strong sinks. This mechanism assures the perennial plant a better survival strategy by expending its resources to support both asexual and sexual reproduction but could result in lower seed set if carbohydrate supplies are limiting.

It has been suggested that the elongating upper internode, following anthesis may compete with developing seeds for available assimilate and adversely affect seed growth (Patrick, 1972; Marshall, 1985). This did not appear evident among control plants in these experiments. Elevated concentrations of carbohydrate (Fig. 3) and evidence of a high degree of fructan polymerization (Griffith, unpubl. res.) observed for control spikelets, suggest that carbohydrate pools appear adequate to support seed growth. In fact, spikelets maintained greater concentrations of carbohydrate than the upper stem. Fructans are known to accumulate when carbohydrate supply is in excess (Pollock and Chatterton, 1988). The upper stem may not be in strong competition with seed filling; rather, it may serve as a temporary storage organ when carbohydrate from the photosynthetically active inflorescence are abundant. The high photosynthetic capacity of the inflorescence of perennial ryegrass may provide much of the photoassimilate for seed growth (Colvill and Marshall, 1984; Clemence and Hebblethwaite, 1984) but supplemented to some extent by stem reserves when carbohydrate supply is limited. The degree to which stem reserves are remobilized appears to be directly related to overall source strength.

When detillering and defoliation were imposed together, carbohydrate reserves were significantly reduced in all stem regions, and in spikelets. The upper stem was unable to accumulate carbohydrate, probably because of reduced photoassimilates and high sink demand of new tillers and seed filling. These findings demonstrate the reliance of the upper stem on post-anthesis photoassimilate for reserve accumulation and later remobilization.

Leaf assimilates were not measured in this experiment so the extent to which carbohydrate was partitioned from leaves is not known. Pulse-chase studies in perennial ryegrass, using $^{14}CO_2$, have shown that during the period of early seed fill, the flag leaf and subtending leaves are important in supplying carbohydrate for seed growth (Clemence and Hebblethwaite, 1984). Later in seed development, the inflorescence and stem appear to contribute a greater proportion of carbohydrate to seeds. In contrast, Colvill and Marshall (1984) have indicated that seed growth in perennial ryegrass appeared to be relatively independent of leaves for current assimilate. The flag leaf and lower leaves partitioned the major portion of ^{14}C to stem internodes, suggesting that leaves were important primarily in providing pre-anthesis carbohydrate to support early inflorescence development. This discrepancy in partitioning patterns between the two studies, as Marshall (1985) suggests, may have been due to differences in nitrogen and water status. Clemence and Hebblethwaite's plants were grown under higher nitrogen and water regimes, conditions of which would support new tiller growth. However, species differences cannot be ruled out.

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